REMARKS

Substitute paragraphs have been provided to correct misspellings in the specification: the word "regimens" on page 3, line 10, and the word "pyrolidone," on page 12, line 25. Thus, no new matter has been added by amendment to the specification.

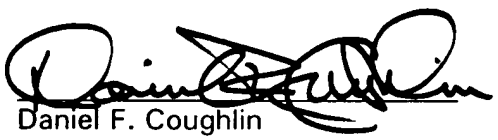
Claims 5-8 are cancelled without prejudice. Further, claims 1, 9, 10, and 19 are amended as indicated. The amendments have been made merely for purposes of clarity, and not for any reason related to patentability of the invention. Claims 28-35 are additional claims pending in the application. Support for the added and amneded claims is found in numerous places in the specification, and in the claims as filed. Accordingly, no new matter has been added by amendment to the claims.

CONCLUSION

Applicant hereby respectfully requests consideration and examination of the currently pending claims.

Date: February 13, 2002

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE TO PARAGRAPHS

Page 3, paragraph beginning at line 6:

Efforts today have centered on improving the survival rates of stored oocytes by improving cryopreservation techniques. According to Martino *et al.* (Martino *et al., Biol. Reprod.* 54: 1059 - 1069 (1996)), such efforts have focused on comparing different cryoprotectants (Otoi *et al., Theriogenology* 40: 801-807 (1993); Dinnyes *et al., Cryobiology* 31: 569 - 570 (1994)) and different freezing *[regiments]* regimens (Lira *et al., Theriogenology* 35: 1225 - 1235 (1991)); or related vitrification methods (Otoi *et al., Theriogenology* 40: 801 - 807 (1993); Otoi *et al., Cryobiology* 37: 77 - 85 (1998)).

Page 12, paragraph beginning at line 17:

Oocytes or embryos are suspended in an equilibration medium consisting of 4% (v/v) ethylene glycol or other intracellular cryoprotective agent in moderate concentration, in a base medium (TCM 199 or similar solutions) supplemented with 20% fetal bovine serum, or bovine serum albumin, or any other macromolecules with surfactant effects at room temperature, or higher, physiological temperatures (39°C for example) for several minutes. Following this equilibration period, groups of oocytes or embryos are rinsed at least two times in small drops of vitrification solution consisting of 35% ethylene glycol (or other intracellular cryoprotectants in high concentration), 5% polyvinyl-*[pirrolidon]* pyrolidone (or other macromolecules), 0.4 M trehalose (or other sugars) in base medium and 20% fetal bovine serum, or other surfactant compounds, as described above, for a few seconds and dropped on the surface of a steel cube, or other solid surface with good heat conductivity, which is cooled down to around -150°C to -180°C or similar subzero temperatures by partially immersing it into liquid or solid nitrogen or into other cooling agents. It

is preferred that the drop size be about 4 μl or smaller, more preferably 3 μl or smaller, and yet more preferably 2 μl or smaller, and yet more preferably 1 μl or smaller, which allows instantaneous vitrification. The vitrified droplets can be moved with a nitrogen-cooled forceps or other tool into 1-ml cryovials or other suitable containers.

VERSION WITH MARKINGS TO SHOW CHANGES MADE TO CLAIMS

1. (Amended) A method for the vitrification of biological materials, said method comprising the steps of:

(a) suspending the biological material in a cryoprotective equilibration [medium] solution, having a concentration of cryoprotectant(s) below that sufficient to protect against ice formation to the glass transition temperature of the cryoprotective equilibration [medium] solution;

(b) rinsing the equilibrated biological material with a vitrification solution, [the vitrification medium] having a concentration of cryoprotectant(s) sufficient to protect against ice formation to the glass transition temperature of the vitrification [medium] solution; and

(c) dropping the vitrification solution-rinsed biological material in the form of discrete microdroplets of vitrification solution, the microdroplets having an average volume of 10 μL or less, onto a substantially stationary solid surface with [good] heat conductivity, as measured at 20° C, of about 10 W/(m-k) [having been] which has previously been cooled [down] to a temperature of about -150° C to about -180° C.

9. (Amended) An improved method for cryopreserving biological material suspended in a vitrification solution, wherein the improvement comprises ccntacting discrete microdroplets having an average volume of 10 μL or less of the vitrification solution containing the biological material with a substantially stationary solid cryogenic surface having a temperature of about -150°C to about -180°C, said surface having a thermal conductivity at 20°C of greater than about 10 W/(m-k) and removing the frozen microdroplets from said surface.

10. (Amended) A method for the vitrification of oocytes, said method comprising the steps of:

(a) suspending the oocytes in a cryoprotective equilibration [medium] solution, having a concentration of cryoprotectant(s) below that sufficient to protect against ice formation to the glass transition temperature of the cryoprotective equilibration [medium] solution;

(b) rinsing the equilibrated oocytes with a vitrification solution, [the vitrification medium] having a concentration of cryoprotectant(s) sufficient to protect against ice formation to the glass transition temperature of the vitrification [medium] solution; and

(c) dropping the vitrification solution-rinsed oocytes in the form of discrete microdroplets of vitrification solution, the microdroplets having an average volume of 10 μL or less, onto a substantially stationary solid surface with [good] heat conductivity, as measured at 20° C, of about 10 W/(m-k) [having been] which has previously been cooled [down] to a temperature of about -150° C to about -180° C.

19. (Amended) An improved method for cryopreserving oocytes suspended in a vitrification solution, wherein the improvement comprises contacting discrete microdroplets containing 10 μL or less of the vitrification solution containing the oocytes with a substantially stationary solid surface having a temperature of about -150° C to about -180 ° C, said surface having a

thermal conductivity 20° C of greater than about 10 W/(m-k) and removing the frozen microdroplets from said surface.

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